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Microbial transformation of Nitrogen in forests

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SUMMARY

The mineralization of nitrogen and the N fixation are investigated in beech and spruce forests in Mirwart (Ardennes belges). Mineralization is well achieved and discreet nitrification can be detected. An heterotrophic germ, *Aspergillus* sp., is able to nitrify at a very low rate. The N fixing capacity of three niches (rhizosphere, litter and phyllosphere) is evaluated and discussed.

RÉSUMÉ

La minéralisation de l'azote et la fixation d'azote atmosphérique sont évaluées dans la hêtraie et la pessière à Mirwart (Ardennes belges). Les protéines sont dégradées avec un rendement satisfaisant et une légère nitrification peut être mise en évidence. Un germe hétérotrophe, *Aspergillus* sp. peut produire de faibles quantités de nitrates. La fixation potentielle d'azote est évaluée et discutée dans trois niches : la rhizosphère, la litière, la phyllosphère.

I. INTRODUCTION

The microflora operates at many stages of the nitrogen cycle. Indeed, the nitrogen mineralization represents an essentiel stage in the supply of mineral N to the plant nutrition as $N\text{-}NH_4$ or $N\text{-}NO_3$. From the point of view of the N balance in a forest, gaseous N fixation can be important where the soil C/N ratio indicates that nitrogen is a limiting factor.

The investigations are therefore focused on those topics and are conducted in acidic soils which are developed under beach and spruce forests in Mirwart (country of Ardennes belges, belgian site of the IBP program). Those forests are fully described by DUVIGNEAUD *and alii* (1972) and DENAEYER-DE SMET and DUVIGNEAUD (1972).

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II. RESULTS

1. N MINERALISATION

Organic nitrogen released on the soil must be decomposed into mineral nitrogen before being assimilated by the plants.

The rate of protein (gelatin) degradation has been followed by burying X-ray films vertically in the soil (DE LEVAL and REMACLE, 1974, PARMENTIER 1974, REMACLE 1974). The rate is estimated to ca 10 g/m^2. year in the soils of beech and spruce forests. But this figure cannot be incorporated directly in the N balance because the amount of degradation depends on the size of the film. So, we have calculated the percentage of decomposition which equals 50 % in a Spruce forest and 64 % in a beach forest (*fig.* 1). The slopes of the curves show that the maximum activity occurs between February and April and between October and November specially in the beech forest. By knowing the concentration of organic N in the litter, it is easy to infer the amount of mineralized N; most of the protein degradation being achieved during the vegetation period. So it can be assumed that 10 kg N/ha.yr are mineralized in a spruce forest and 32 kg N/ha.yr in a beech forest (REMACLE 1974).

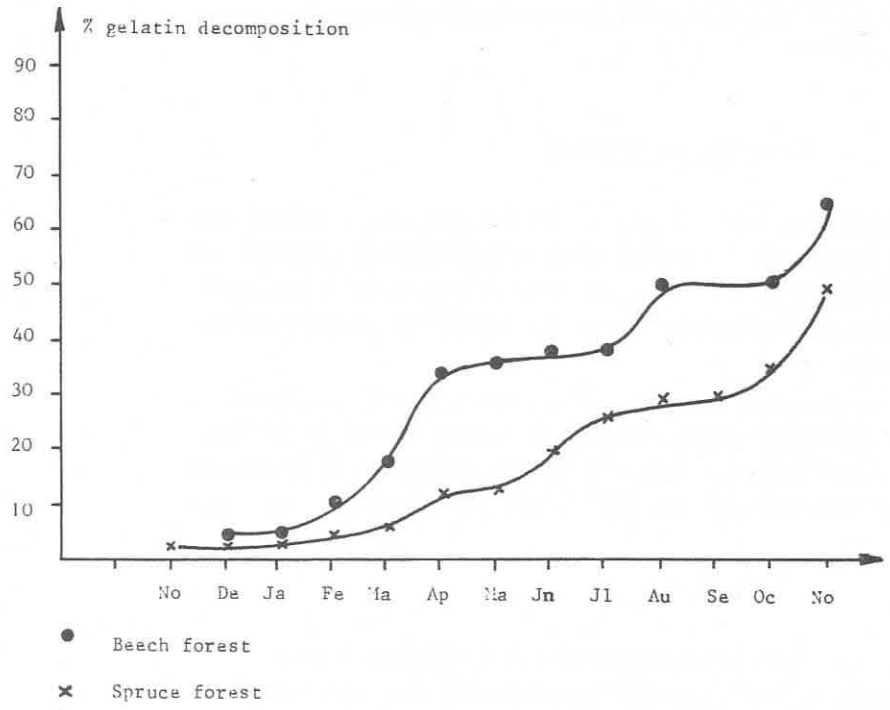

FIG. 1. – Rate of gelatin decomposition in beech and spruce forest soils.

The proteolytic microflora is composed mainly of bacteria (*Pseudomona* sp.) in beech forest soils and of fungi in spruce forest soils. As it has been observed that the growth of a proteolytic germ (*Flavobacterium* sp.) releases mainly $N–NH_4$ (COUMONT 1975), it can be assumed that most of the mineralized N is $N\text{-}NH_4$, the amount of N immobilized in microbial cells being unimportant (8 to 15 %).

2. NITRIFICATION

By *in situ* and *in vitro* observations, FROMENT and REMACLE (1975) have showed that nitrification occurs at a low rate in the spruce forest soils. In those soils, 6.4 mg $N\text{-}NO_3/m^2$.day are produced by incubation. VAN PRAAG *and alii* (1972) have noted the same process in beech forest soils.

Those very acidic soils do not promote the development of autotrophic nitrifying germs as proven by microbiological analysis.

By continuous cultures, a heterotrophic nitrifying germ has been isolated; it belongs to the genus *Aspergillus*. It is mesophilic (*fig.* 2). The production of $N\text{-}NO_3$ vs pH is evaluated in continuous culture. The composition of the medium is as follows: peptone 0.1 g, glucose 0.1 g, Winogradski's solution 50 ml, buffer and H_2O q.s.p. 1 000 ml. The pH varies from 6 to 9.5. Cultures are incubated at 23 °C and the dilution rate is maintained around 0.02 h^{-1}. The mycelium production is also evaluated.

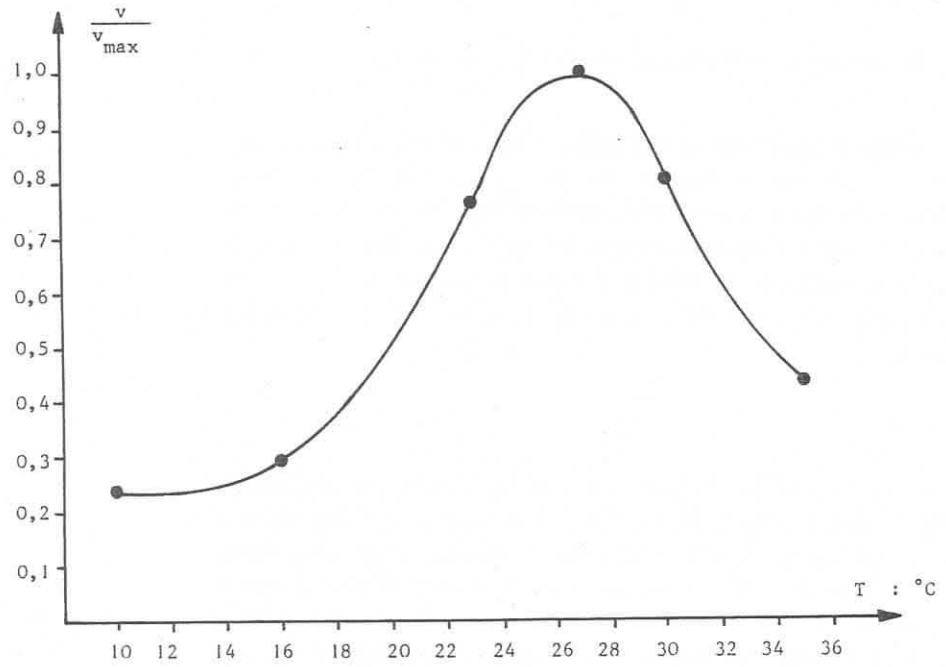

FIG. 2. – Mycelium productivity vs temperature.

As shown in figure 3, it is rather surprising to note that the optimal pH for $N\text{-}NO_3$ production is not the same as for mycelium production.

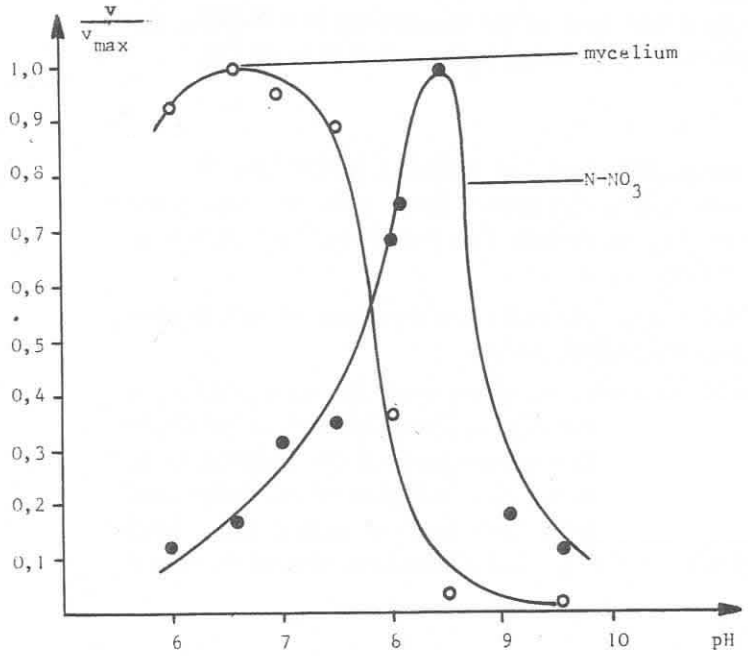

FIG. 3. — Rate production of $N\text{-}NO_3$ and mycelium by Aspergillus sp.

The optimal pH settles respectively at 8.3 and 6.5 for $N\text{-}NO_3$ and mycelium production. The strain is slightly acidophilic on the ground of the mycelium production. The maximum production of $N\text{-}NO_3$ reaches 16 µg $N\text{-}NO_3$/1.h. and the maximum production of mycelium equals 5.6 mg/1.h. The yield is very low because 0.1 % of N-peptone is converted into $N\text{-}NO_3$ in the best conditions (0.01 % in the worst conditions). The production of $N\text{-}NH_4$ is always very low except, at pH 8.3, where it reaches 10 µg/1.h.

3. N FIXATION

The estimation of the N fixation is best achieved by the *in situ* method as devised by BALANDREAU and DOMMERGUES (1971). But before applying such a technique, it is worth detecting the niches where the N-fixation is possible. Since N fixation needs a lot of energy, it can occur only in niches where energetic substrates are available. Three niches are investigated : the rhizosphere, the litter and the phyllosphere. No symbiotic N fixation is observed in these forests. As could be expected, no aerobic N-fixing germs are isolated in the acidic soils. But anaerobic

bacteria colonize the horizons of the beech and spruce forests specially in the rhizosphere of *Festuca altissima* All. in the *Festuca* beech forests.

TABLE 1

Anaerobic N fixing germs in forest soils (10^3 g × g.d.w^{-1})

Forest	March	June	September	November
Top beech forest	1.2	33	0.1	9
Festuca beech forest	34	5	21	50
Festuca rhizosphere	23	230	143	17
Spruce forest	0.08	0.6	0.6	7

Thus, rhizosphere seems to be a niche where N fixation could be significant.

Litters also form a niche which can provide available organic matter. In the experiment, the L and F layers were sampled in the top beech and *Festuca* beech forest and they were incubated, during 8 months, in a ventilated incubator under the following conditions : temperature 18-20 °C, air moisture 80-90 %, darkness. The following formula is used (REMACLE and VANDERHOVEN, 1973) in order to know, at the end of incubation, the initial N content which has increased falsly by Carbon respiration :

$$N_2 = N_1\left(\frac{100 - C_2}{100 - C_1}\right)$$

N_2 : theoritical N content at the end of incubation
N_1 : N content at the beginning of the incubation
C_1 : C content at the beginning of the incubation
C_2 : C content at the end of the incubation.

The difference between the measured N content at the end of incubation and N_2 equals the N fixation.

TABLE 2

N fixation in L and F layers in beech forests (mg × g.d.w.$^{-1}$ × month^{-1})

		Loss of C	$\frac{C_1}{C_2}$	Increase
Top beech forest	L	5.6	1.09	0.57
	F	6.3	1.12	0.82
Festuca forest	L	6.1	1.10	0.46
	F	3.6	1.07	0.31

This experiment leads to the following conclusions :

Firstly, there is N fixation and the best rate of N fixation is restricted to the F layers of the top forest (*Fagetum nudum*).

Secondly, there is a good correlation (R = 0.91) between the decomposition index $\frac{C_1}{C_2}$ and the N fixation (Table 2, *fig.* 4).

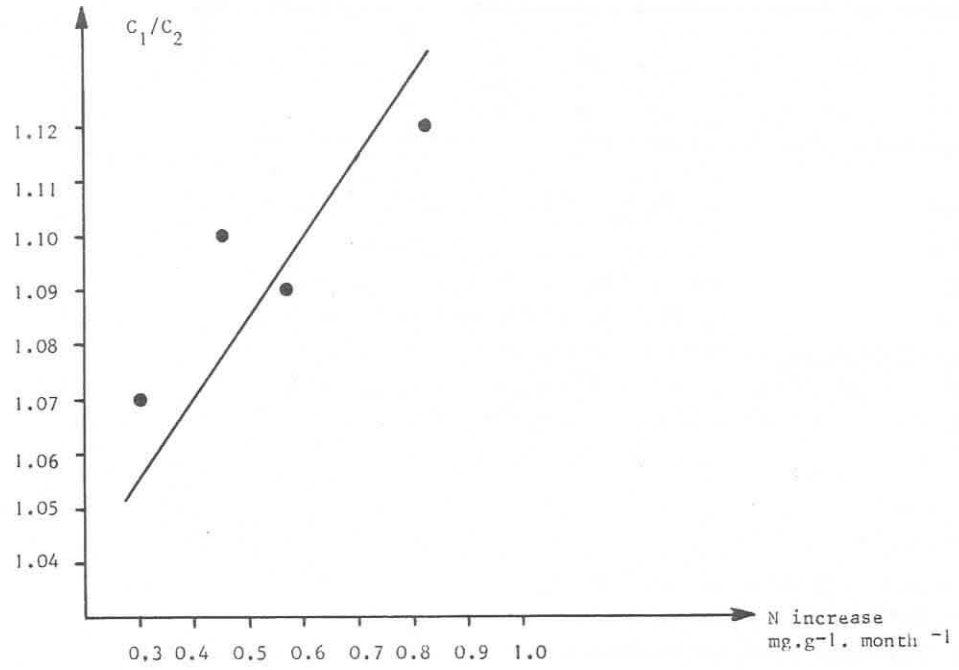

FIG. 4. – The N fixation vs the decomposition index.

Lastly it can also be noted that the mean ratio $\frac{C_1}{C_2} = 1.09$ is rather similar to the Ledel's « decomposition index » (LEDEL 1973) which has been calculated *in situ* for the same forests : $\frac{L}{LS} = 1.32$ (mean value).

L : leaves fallen in 1971
LS : litter in 1972.

These conclusions warrant the use of the relation between C consumption and N fixation in order to evaluate the potential N fixation in the beech forest; more especially as no blue-green algae has been observed in litter by the usual technique (AARONSON 1970).

We shall adopt the yield coefficient of ALEXANDER (1967) : 0.5 % similar to the ABD-EL-MALEK's one (1971) for they have been calculated for field conditions. If we assume that LS equals the litter fall for the preceding year, the loss of substrate due to decomposition can be calculated. In this case, $L - LS = \left(\frac{1.32 - 1}{1.32}\right) L$. Since L equals 3 000 kg/ha.yr (DUVIGNEAUD *and alii*, 1972), L-LS is equivalent to 722 organic matter kg/ha.yr. From this figure, it can be estimated that 3.6 kg N/yr.ha could be fixed.

Many organic compounds are released by leaves in the phyllosphere (MANGENOT, 1966) which is colonized by a rich microflora and particularly by N fixing germs (RUINEN, 1965). The potentiality of N fixation in this niche is checked as follows. The microbial population of a known dry weight of leaves is precultured in a N free medium in order to enhance the development of N-fixing germs. After 8 days, the precultures are then transferred into 50 ml of a N free medium, supplemented with soil extract. The incubation is conducted at 20 °C on an agitator during 2 months. At the end of incubation, the amount of fixed N is determined bearing in mind the N content at the beginning of the experiment.

TABLE 3

N fixation by the phyllosphere microflora

		Experiment (mg N . g.d.w. of leaves^{-1} . month^{-1})	Forest (g N . ha^{-1} . year^{-1})
Beech	Leaves	0.225	4.7
Spruce	Young needles (1 year)	0.170	2.7
	Old needles	0.037	

When it is based on the dry weight of leaves, the best yield is obtained by the beech leaves and the old needles of Spruce are characterized by the lowest N-fixing capacity. The N fixation for the vegetation period (7 months) would reach 4.7 kg in beech forest and 2.7 kg in Spruce forest for the dry weight of leaves equals 3 000 kg/ha.yr in the beech forest and the dry weight of the young needles reaches 2 270 kg/ha.yr in the Spruce forest.

DISCUSSION AND CONCLUSIONS

The proteins are mineralized at nearly the same rate in beech and spruce forest (64 % and 50 %). The amounts of mineral N calculated from these percentages underestimate the amounts which have been evaluated by incubation

techniques. Thus, VAN PRAAG *and al.* (1973) observe a production of 107 kg N/ha.yr in beech forest (32 kg N/ha.yr from the rate of gelatin decomposition) and FROMENT and REMACLE (1974) conclude by *in vitro* incubations that 34 kg N/ha.yr are mineralized in Spruce forest (10 kg/ha.yr from the rate of gelatin decomposition). In the spring, there is a good correlation between the increase of $N\text{-}NH_4$ and the development of ammonifying germs (FROMENT and REMACLE 1974). A very discrete nitrification is detected by incubation method (FROMENT and REMACLE 1974).

An acidophilic germ, Aspergillus sp., can nitrify at high pH (8.4) which confirms the observations of VERSTRAETEN and ALEXANDER (1973). The maximal production is low in regard to other heterotrophic productions (ALEXANDER 1967, DOMMERGUES and MANGENOT 1972) and, of course, to autotrophic production in pure culture and even, in mixed culture (DE LEVAL and REMACLE 1975).

But, if the $N\text{-}NO_3$ production is related to the mycelium production, the observed production 28.5 mg $N\text{-}NO_3$/h.g.d.w. is higher than the VAN GOOL and SCHMIDT's results (1973) : ca 5.0 mg $N\text{-}NO_3$/h.g.d.w. These authors have studied also an *Aspergillus* strain in the same type of medium. As it concerns $N\text{-}NH_4$ production, they observed that the $N\text{-}NH_4$ yield is lower than the $N\text{-}NO_3$ yield which is confirmed by our experiments.

Rhizosphere and phyllosphere excretions have been characterized many years ago (VANCURA 1964, MANGENOT 1966). Young litters contain also high concentration of organic material. In the rhizosphere, it has been noted that only anaerobic N-fixing germs are detected, which confirms the observations of KNOWLES (1965). If the potential N-fixing capacity of litter and phyllosphere are compared it can be concluded that litter and phyllosphere can fix the same amount of gaseous N. Despite the very low rate of N-fixation, the yield of N- fixation in litter seems to be rather high if compared to the values of the literature (POCHON and DE BARJAC, 1948, ALEXANDER 1967, DOMMERGUES and MANGENOT 1972) but the litters are incubated in favorable conditions. The N supply to forest by litter fixation ranges around 3.6 kg/ha.yr which agrees with the results of BALANDREAU *and alii* (1973). These authors use the *in situ* acetylene method and evaluate the N-fixation to 1-5 kg N/ha.yr in the same type of forests.

The rate of N fixation in the phyllosphere is rather consistent with field observations. Thus, JONES (1970) has pointed out that the phyllosphere microorganisms are able to fix between 20 and 64.5 g N/ha.day in DOUGLAS fir forest, i.e. 600 to 1 935 g/ha.month which is as high as the amounts we measured. Our results show also that the N fixing capacity depends upon the age of the needles. This observation puts in light the difficulty to obtain a accurate assessment of the *in situ* N fixation because, the localization and the microclimate of the niche will influence greatly the rate and the yield of the process. At last, by adding all the possible N fixation mechanisms, it should be feasible to know the weight of this process upon the N balance.

In the case of MIRWART beech forest, phyllosphere and litter can contribute to an increase of 8.3 kg N/ha.yr, no figure is available for the rhizosphere. By including the rhizosphere, the N increase would be ca. 10-15 kg N/ha yr which is rather low in comparison with the N amount in forest : ca. 500 kg N/ha. (DENAEYER *and al.* 1972).

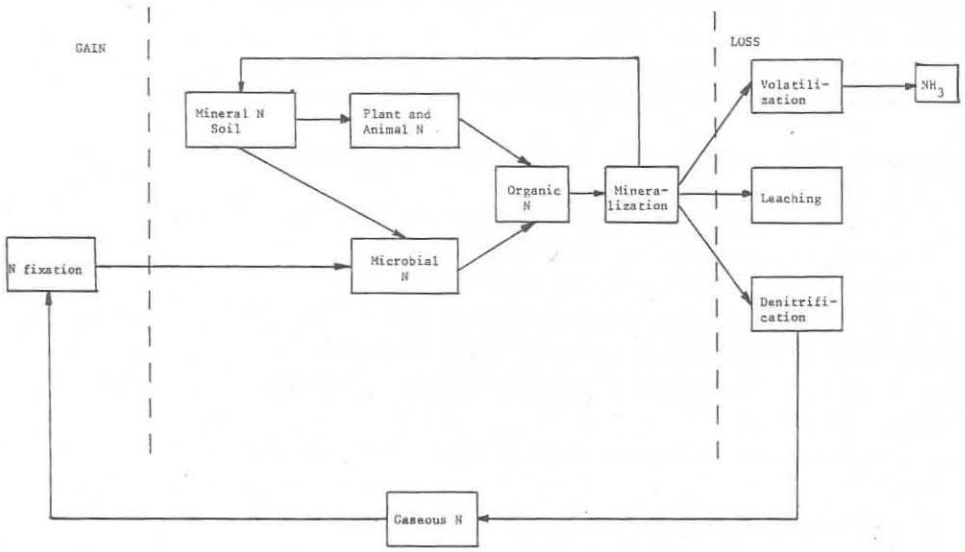

FIG. 5. – Microbial N balance in forest soils.

Besides if a block-diagram of the microbial N balance in forest, is conceived on the grounds of profit and loss by microbial activities (*fig.* 5), it is obvious that for an accurate N balance, N fixation will be put in regard to possible losses : leaching (enhanced by microbial chelating compounds) denitrification (in anaerobiosis) or ammonia volatilization (MAHENDRAPPA and SALONIUS 1974). Of those three processes, denitrification and leaching are the most probable. Finally it means that N-fixation could not influence greatly the N balance in these forests.

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